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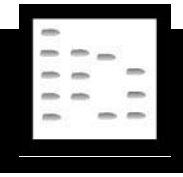
POLYETHERS: LIQUID CHROMATOGRAPHY



K. Rissler, Ciba Specialty Chemicals, Basle, Switzerland

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Introduction

Before chromatography became an efficient tool for polymer fractionation, classic extraction procedures played a dominant role. These techniques comprise, e.g., dissolution and precipitation, depending on the solvent–non-solvent ratio and M_r, extraction of native polymer or polymer adsorbed onto a solid support with organic solvents of increasing dissolution capability, and partition between two immiscible liquids. In contrast, Baker–Williams and temperature rising elution fractionation (TREF), also used in polymer chemistry, are chromatographic techniques. Although separation of polymers by means of various classic extraction procedures are still in use, chromatographic characterization now plays the dominant role and affords an optimum degree of structural information. In this respect, polyethers of the polyethylene glycol (PEG), polypropylene glycol (PPG) and polybutylene glycol (PBG) family, all extensively used in different fields of chemistry and engineering, have been selected as model compounds for separation of polymers because they differ widely in chemical properties and polarity, ranging from hydrophilic (PEGs) to hydrophobic (PBGs) in either native form or mono-(di-)O-alkyl(arylalkyl) (**Figure 1**A–C) and amino-terminal derivatives (**Figure** 1B). For this reason, they comprise a group of polymers accessible to a broad range of chromatographic separation techniques including high performance liquid chromatography (HPLC), size-exclusion chromatography (SEC), thin-layer chromatography (TLC), supercritical fluid chromatography (SFC) and capillary zone electrophoresis (CZE). Gas chromatography (GC) only provides separation of the low-molecular-weight (M_r) members of polyethers with upper limits of M_r of approximately 600. TLC and SFC are of minor importance and are not considered. Although extensively used for determination of M_r values, SEC is also excluded because it exhibits only moderate resolution and does not permit differentiation of the individual types of polyether on the basis of the underlying chemistry.

This survey gives a short overview of the current state of HPLC technology of polyethers and deriva-

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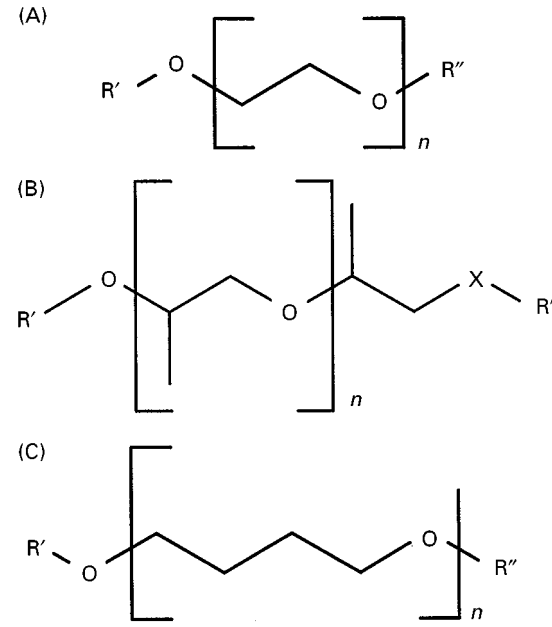

Figure 1 Structures of polymers. (A) R′, R″ = H, n-alkyl, alkyl–C=O, aryl(alkyl); R′ = R″, R′ ≠ R″; (B) R′ = H, glyceryl, trimethylolpropyl; R″ = H (X = O), H_2 (X = N); (C) R′ = R″ = H, alkyl, aryl(aroyl); R′ = R″, R′ ≠ R″.

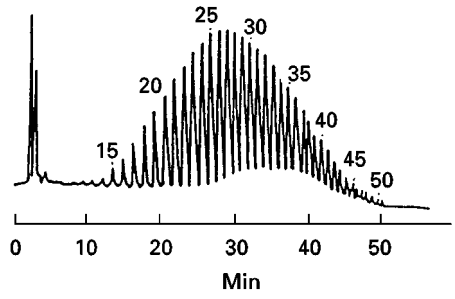

Figure 2 Gradient elution of the oligomers from NPE. Eluent A, n-hexane–2-propanol (40 : 60, v/v); eluent B, ethanol–water (80 : 20, v/v). Gradient: 10–95% B in 45 min. (Reproduced from Anghel DF, Balcan M, Voicu A, *et al.* (1994) *Journal of Chromatography*, 668: 375–383, with permission from Elsevier Science.)

tives. For more detailed information on liquid chromatography of this class of substances the reader is referred to recently published review articles (see Further Reading).

Special Features of Chromatographic Separation of Polymer Systems

Chromatographic behaviour of synthetic polymers substantially differs from that of low-molecular-weight analytes for a variety of reasons: (i) small diffusion coefficients of macromolecules in solution, (ii) the size of the macromolecules often being of the same order of magnitude as the pore diameter of the stationary phase, (iii) retention of polymers via trains of numerous repeat units, (iv) flexibility of the polymer chains enabling conformational changes, and (v) limited solubility. Synthetic polymers are usually composed of a large number of identical structural units, which dependent on the experimental conditions, give rise to more or less broad M_r distribution, ranging from some hundreds to millions. The situation is still more complicated when statistical, random and block copolymers are formed. Isocratic HPLC, typically applied for low M_r analytes, is not suited for exhaustive separation of individual oligomers, because of the great number of molecules often differing greatly in M_r. Separation of polymers built up from different chemical units (e.g., copolymers and/or polymers containing different end groups, such as, e.g., non-ionic surfactants) should ideally be effected according to either degree of polymerization expressed by the number of repeat units n and thus, M_r weight distribution, or chemical composition (CCD) and functionality type distribution (FTD). However, unfortunately, only in rare cases are both aims achievable within a single chromatographic run and therefore, so-called two-dimensional chromatography is required.

Furthermore, in a much more pronounced manner than for low-molecular-weight analytes, conformational effects must be considered appropriately taking into account distinct structure-dependent intramolecular interactions invoked by both nonpolar (e.g., hydrophobic = van der Waals) and polar (e.g., hydrogen bonding, dipole–dipole, ionic, etc.) interactions. All these effects are responsible for the often

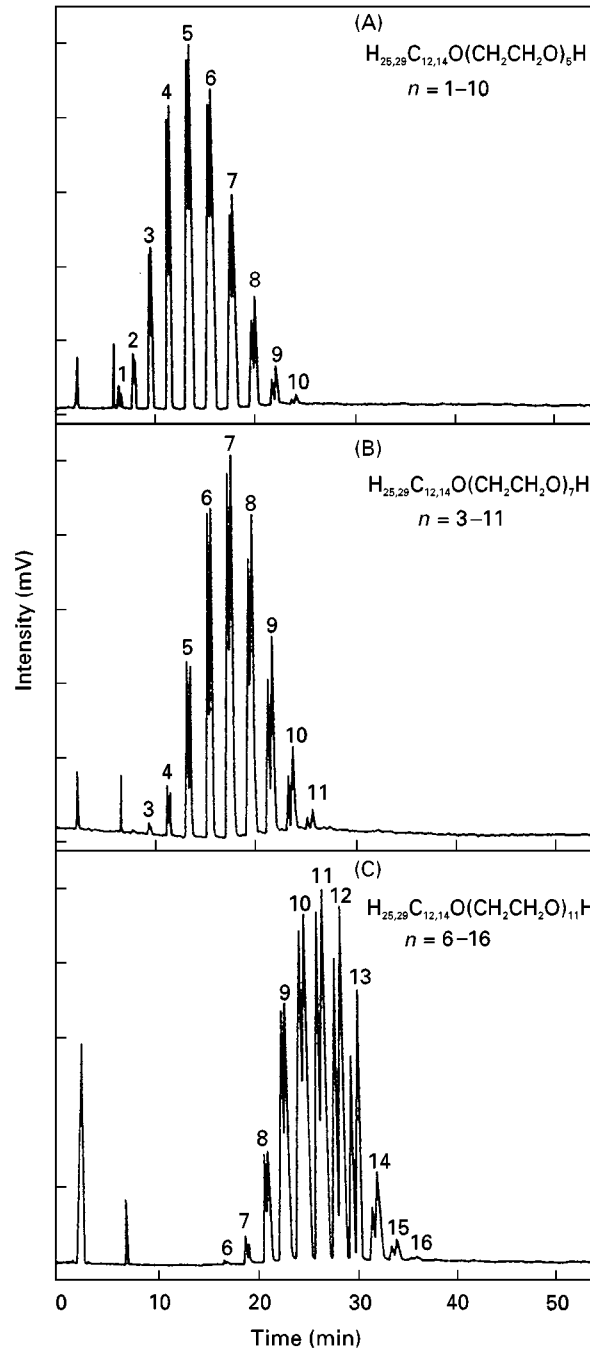

Figure 3 HPLC analysis of linear alkylethoxyalcohol oligomers: (A) AE5; (B) AE7: (C) AE11. (Reproduced from Bear GR (1988) *Journal of Chromatography* 459: 91–107, with permission from Elsevier Science.)

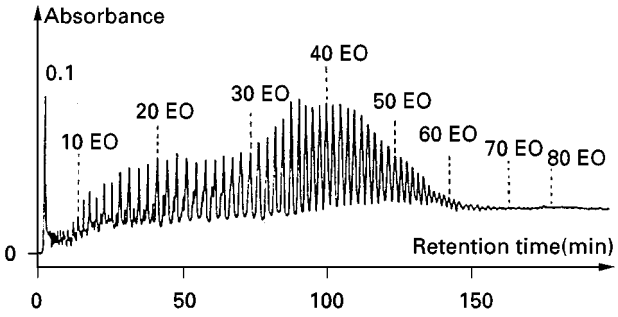

Figure 4 Analysis of a mixture of non-ionic polyoxyethylene (POE) surfactants KM25 (10^4 ppm, w/w) and Cetalox AT (10^4 ppm, w/w) as esters by normal-phase partition chromatography. (Reproduced from Desbène PL and Desmaizières B (1994) *Journal of Chromatography* 661: 207–213, with permission from Elsevier Science.)

unexpected chromatographic properties more or less substantially deviating from the analyte's intrinsic hydrophobicity based on calculation of hydrophobic increments. This behaviour takes into account the phenomenon of multiple attachment of the polymer chain, which means that adsorption onto the stationary phase surface is not effected by participation of the whole macromolecule but attributable to alternating trains of repeat units, whereas a substantial part of the molecule still extends into the surrounding mobile phase. Moreover, the flexibility of the chains easily allows conformational changes, which are further influenced by the gradual change in mobile phase composition during gradient chromatography.

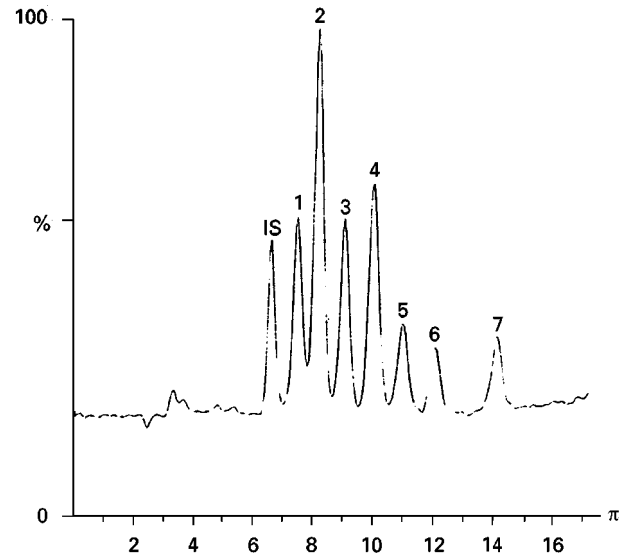

Figure 5 LC/MS chromatogram obtained by injecting the composite working standard solution containing NPEOs and alkylethoxylates (AEOs). The analytes in this synthethic mixture are, in order of elution: 1, NPEOs; 2, C-12 EOs; 3, C-13 EOs; 4, C-14 EOs; 5, C-15 EOs; 6, C-16 EOs; and 7, C-18 EOs. IS (internal standard), C-10 EO_6. (Reproduced from Crescenzi C, Di Corcia A and Samperi R (1995) *Analytical Chemistry* 67: 1797–1804.)

High Performance Liquid Chromatography of Polyethers

Normal-Phase Liquid Chromatography (NP-HPLC)

NP-HPLC is defined as separation on polar stationary phases using pure organic eluents of increasing polarity. For this reason, either bare silica gel or so-called bonded-phase materials, such as 3-cyanopropyl- (CN), 3-aminopropyl- (NH_2) as well as 2,3-dihydroxypropyl (Diol) silica gel are used as solid supports for the separation of fatty alcohol and fatty acid polyethoxylates, the lower M_r members of native PEG and the corresponding octyl- and nonylphenol derivatives. In general, separation occurs with respect to the number of ethoxymers, although side-chain isomerism as well as differences in the length of the alkyl substituent is also observed in some cases yielding substantial splitting of the oligomer peaks. In contrast, polypropylene (PPG) and polybutylene glycols (PBG), possessing a substantially more hydrophobic polymer backbone, are better separated by reversed-phase HPLC (RP-HPLC). Whereas combinations of pure organic solvents often containing small amounts of water to accelerate the adsorption–desorption equilibrium are used on bare silica, the NH_2, CN and Diol materials are also run under typical RP-HPLC conditions with aqueous organic eluents. Although native silica gel is still used for PEG derivatives, it is being replaced more and more by

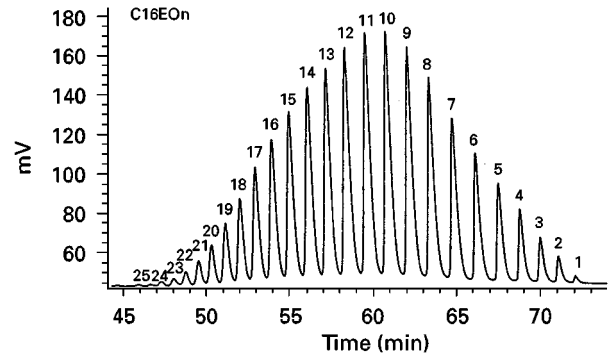

Figure 6 Separation of ethoxylated hexadecanol C16EO (average ethoxylation degree = 10) on reversed phases (stationary phase: Nucleosil 120-3 C18 (250 × 4); mobile phase: A-H_2O, B-CH_3CN, 1 mL min^{-1}. Gradient programme: 46 → 55% B/20 min, 55 → 76% B/30 min, 76 → 90% B/15 min; ELSD–50 mm N_2, 110°C). (Reproduced from Miszkiewicz W and Szymanowski J (1996) *CRC Critical Reviews in Analytical Chemistry* 25: 203–246.)

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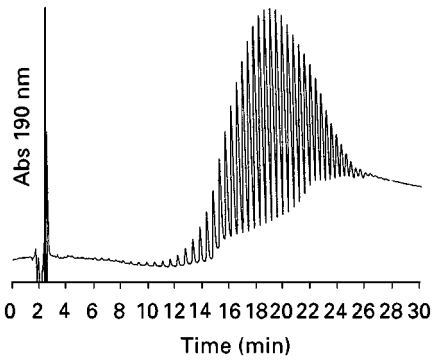

Figure 7 HPLC analysis of PEG with an average molecular weight of 2000 daltons carried out on two ODS columns at 60°C and with a detector sensitivity of 0.2 a.u.f.s. (Reproduced from Escott REA and Mortimer N (1991) *Journal of Chromatography* 553: 423–432, with permission from Elsevier Science.)

CN, NH_2 and Diol matrices, which behave as deactivated silica and thus allow a better fine-tuning of the chromatographic conditions. In addition, PEGs with $M_r > 2000$ are irreversibly retained on bare silica gel matrices and thus should be separated by RP-HPLC. It should be emphasized that the bonded-phase materials are not as sensitive towards traces of water in organic solvents compared with silica gel and retention times and peak shapes exhibit excellent reproducibility making them suitable for long-term application without marked loss of chromatographic performance.

Although isocratic NP-HPLC yields satisfactory resolution of PEGs with lower degree of oligomerization, the higher oligomers are often truncated and merge more and more with the baseline. This drawback can easily be overcome by the solvent gradient technique, which is the predominant method for separation of polymers. Usually, chormatography starts with a mobile phase of low polarity, such as, e.g., n-hexane, n-heptane, isooctane, cyclohexane, etc., and methanol, ethanol, acetonitrile, 2-propanol, tetrahydrofuran (THF), dioxane, etc., are used as the polar modifiers. Due to insufficient miscibility when using methanol and acetonitrile in combination with alkanes, a third component affecting solvent compatibility is required and THF, dichloromethane and chloroform have been successfully applied. In contrast, ethanol and 2-propanol being more lipophilic compared to methanol and acetonitrile, dissolve in aliphatic and cycloaliphatic solvents at any volume ratio. In particular when using the most polar silica gel, elution is often started with a significant percentage of solvents with intermediate polarity like dichloromethane, chloroform or diethyl ether instead of pure alkane and thus, no additional compatibility modifier is necessary. Nevertheless, a third eluent is often used for chromatographic fine-tuning.

Excellent oligomer resolution of octylphenyl- (OPEO), nonylphenyl- (NPEO) and alkyl-ethoxylates is achieved on bare silica gel (**Figure 2**), CN, NH_2 (**Figure 3**), *p*-nitrophenyl-bonded silica (**Figure 4**) and Diol matrices.

Sometimes peak-splitting of the signals of the individual NPEO oligomers occurs, revealing structural heterogeneity within the nonyl side chain, which is

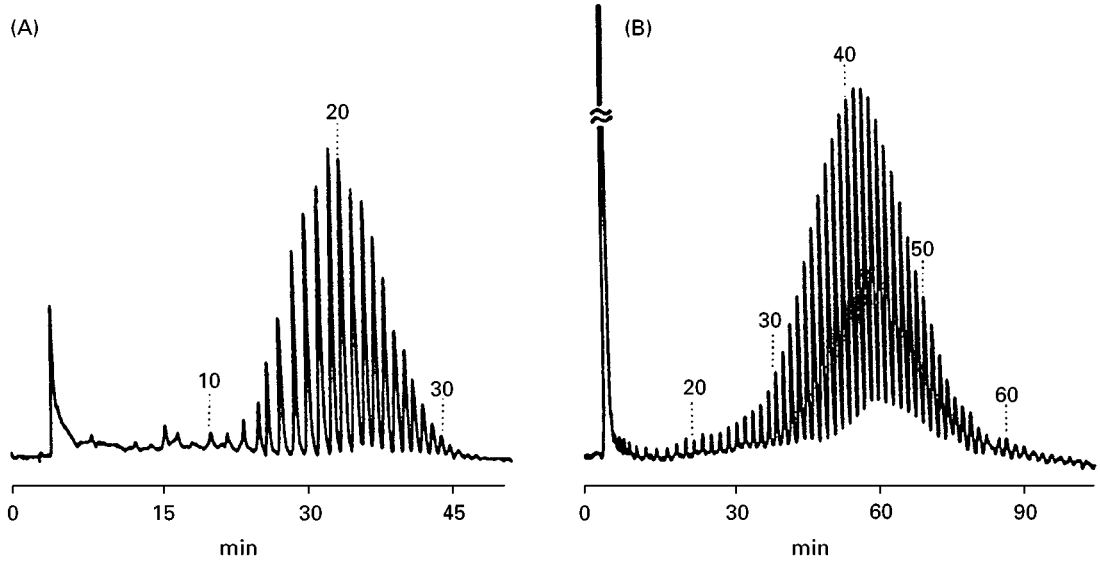

Figure 8 Chromatograms of 3,5-dinitrobenzoyl derivatives of PEGs by HPLC. Conditions: column. 5 μm Spherisorb NH_2 (250 × 4.6 mm I.D); detection, UV at 276 nm (A) PEG 1000, (B) PEG 2000. (Reproduced from Sun C, Baird M and Simpson J (1998) *Journal of Chromatography A* 800: 231–238, with permission from Elsevier Science.)

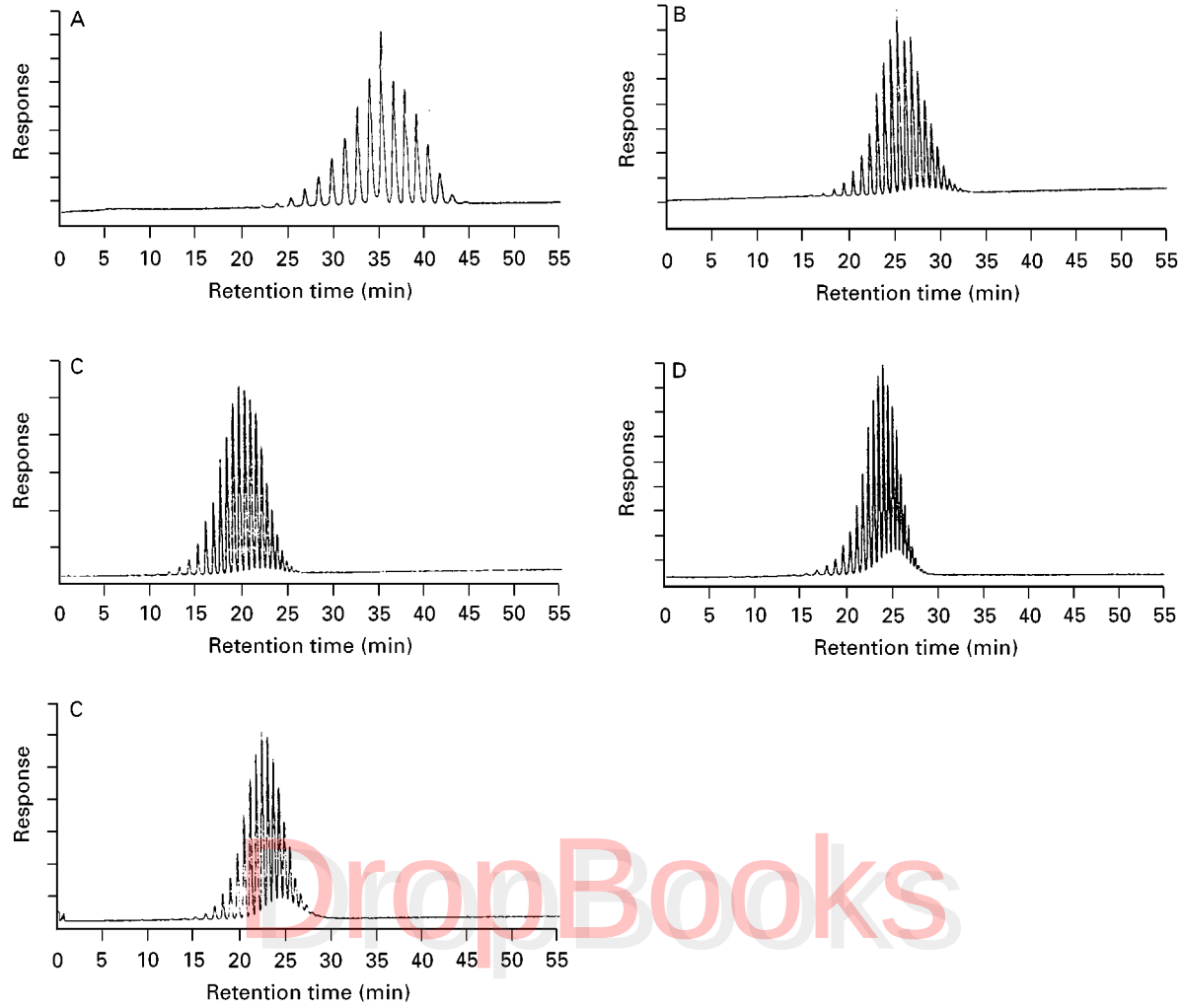

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Figure 9 Chromatograms with PPG-1200 and acetonitrile as organic solvent. (A) C_{18}; (B) C_8; (C) C_4; (D) C_{phenyl}; (E) C_1. (Reproduced from Rissler K, Künzi H-P and Grether H-J (1993) *Journal of Chromatography* 635: 89–101, with permission from Elsevier Science.)

not observed in OPEOs. In the same way separation according to the number of ethoxymer (EO) units as well as the chemical composition of the fatty alcohol chain of nonionic surfactants (NIS) is achieved on an NH_2 stationary phase (**Figure 3**). Nevertheless, in most cases information with respect to both ethoxymer distribution and chemical composition of the alkyl substituent is unsatisfactory and thus RP-HPLC is required for differentiation of the alkyl end groups. Due to the increasing interactive surface of the PEG derivatives with increasing number n of repeat units, retention time of each oligomer increases with M_r, whereas in contrast, the lipophilic end group plays an only marginal role.

In the case of OPEOs and NPEOs bearing an aromatic moiety, signal monitoring is easily accomplished by both UV and fluorescence detection (FD), whereas evaporative light scattering detection (ELSD) is preferred for the alkylpolyethoxylates (Figure 3). However, in gradient elution with varying concentration of a modifier more or less absorbing in the lower UV range, such as, e.g., ethyl acetate, THF, etc., it is advisable to use wavelengths ≥ 250 nm to keep the baseline drift as low as possible. Polyether derivatives lacking a chromophor, such as fatty alcohol or fatty acid polyethoxylates, are amenable to UV detection after conversion to their benzoyl, 3,5-dinitrobenzoyl and phenylurethane derivatives, which in general, give high yields. Furthermore, for extremely sensitive measurement, the hydroxy functions of the polyether samples can be reacted with fluorophors providing high quantum yields, like fluorenylmethoxycarbonyl chloride, 1-naphthylisocyanate, 1(2)-naphthoyl chloride, carbazol-9-carbonyl chloride, 1(9)-anthroylcyanide, etc. Whereas derivatizations with isocyanates run uncatalysed at about 60°C within 1–2 h, the acyl chlorides require base catalysis (e.g., pyridine, triethylamine,

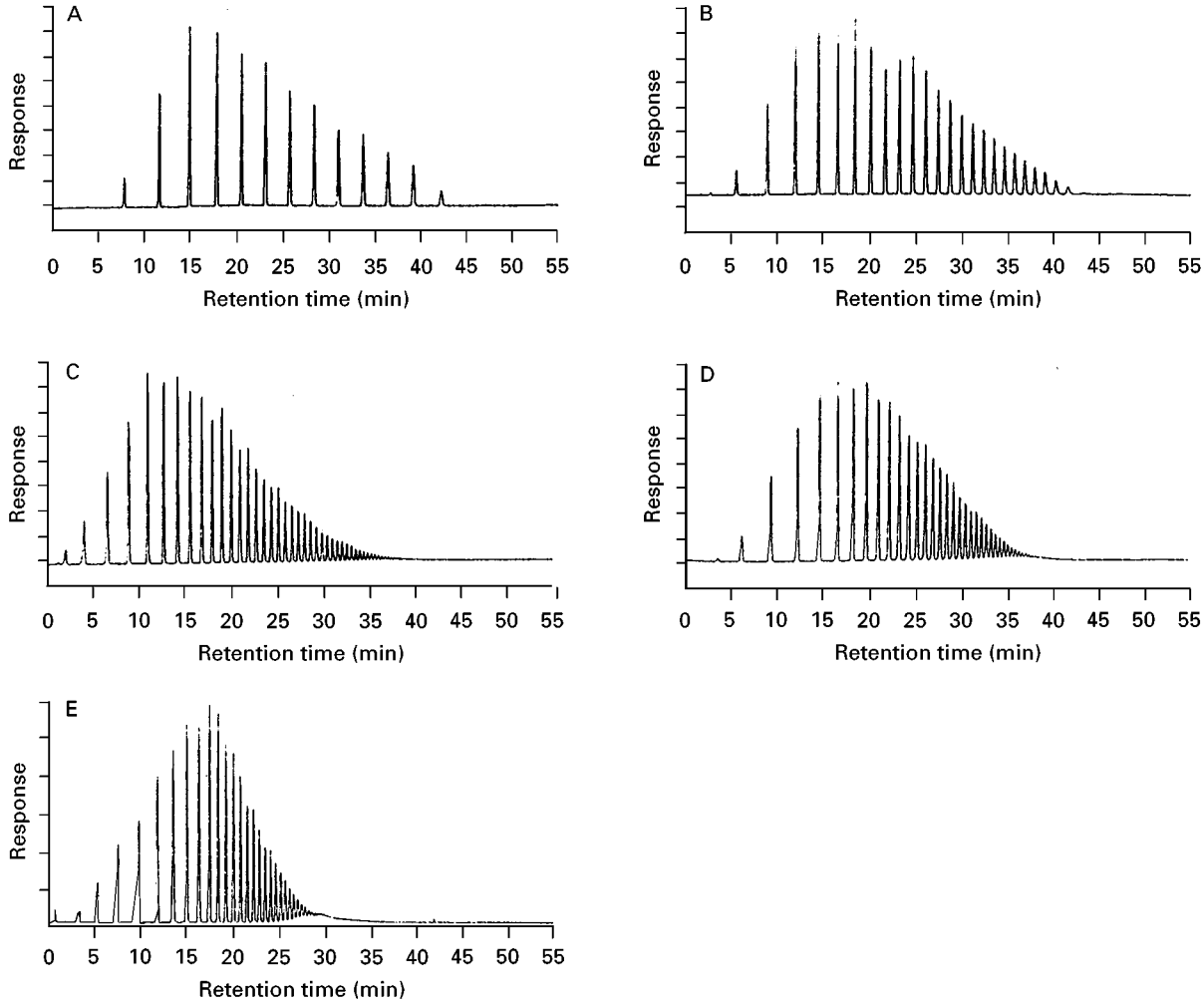

Figure 10 Chromatograms with PBG-1000 and acetonitrile as organic solvent. (A) C_{18}; (B) C_8; (C) C_4; (D) C_{phenyl}; (E) C_1. (Reproduced from Rissler K, Künzi H-P and Grether H-J (1993) *Journal of Chromatography* 635: 89–101, with permission from Elsevier Science.)

methylimidazole, etc.). In the latter cases either heating for about 0.5–1 h or working at room temperature for about 0.5–2 h generally affords good yields.

Reversed-Phase Liquid Chromatography (RP-HPLC)

RP-HPLC is effected on hydrophobic stationary phases using eluents of decreasing polarity. In general, it is the method of choice for separation of PEG-based nonionic surfactants (NIS) with respect to the chemical structure of the hydrophobic end groups without separation according to the degree of ethoxylation (**Figure 5**) and also PEGs, PPGs and PBGs. In most applications octadecasilyl silica gel (C_{18}) and/or octylsilyl silica gel (C_8) stationary phases with binary gradients of acetonitrile (methanol)–water are used.

Separation of alkylpolyethoxylates according to the number of ethoxylate units with an acetonitrile–water gradient on a C_{18} column has also been reported (**Figure 6**) and retention increases with decreasing number of ethoxymers. Surprisingly, the elution order of oligoethylene glycol phenyl (octylphenyl) ethers is reversed in mobile phases of methanol and water.

Although being significantly less hydrophobic than their corresponding NIS counterparts, satisfactory separation of PEGs is achieved for the species up to $M_r \cong 2000$ using a C_{18} column, whereas in general, PEGs with $M_r \geq 2000$ merge more and more into a common signal envelope. **Figure 7** reveals separation of PEG 2000 by use of two C_{18} columns in series.

Despite the fact that separation of PEGs according to the degree of ethoxylation decreases substantially at $M_r > 2000$, PEGs widely differing in M_r ranging from some hundreds to some hundred thousands can be efficiently separated from each

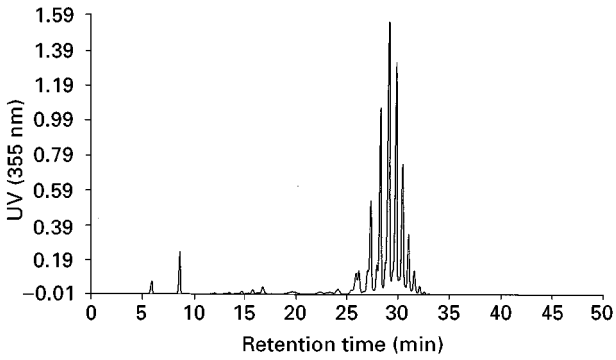

Figure 11 RP-HPLC of Jeffamine D 400™ derivatized with 2,4-dinitroflurorobenzene on a Nucleosil 5C18 column (125 × 4.6 mm; 5 μm) with a gradient from water to 65% acetonitrile–10% THF at 25 min, 80% acetonitrile–20% THF at 40 min, 80% acetonitrile–20% THF for 10 min followed by a drop to the starting conditions and re-equilibration for 14 min at a flow rate 1.5 mL min^{-1}; detection wavelength 355 nm. (Reproduced from Rissler K (1998) unpublished results.)

other as single peaks using binary acetonitrile (methanol)–water gradients and C_{18} or C_8 stationary phases. In contrast, bonded phases, such as CN and Diol materials, run under RP-HPLC conditions, are less suited for efficient separations of PEGs with $M_r \leq 2000$ into their oligomers. The exception is an NH_2 matrix providing excellent separation of PEGs up to M_r of about 2000 as their 3,5-dinitrobenzoyl derivatives with a binary gradient of acetonitrile and water (**Figure 8**).

As a consequence of the substantially better interaction of their more hydrophobic backbones with nonpolar stationary phases, PPGs (**Figure 9**) and PBGs (**Figure 10**) are much better resolved into individual oligomers and often baseline separation is achieved.

PPGs up to $M_r \cong 2000$ can be sufficiently separated with binary acetonitrile–water gradients, whereas the higher M_r oligomers elute as more or less broad but unresolved signals. At $M_r > 2000$ it becomes more and more difficult to achieve complete elution of either native PPGs or their amino-terminal derivatives from a highly hydrophobic C_{18} matrix with binary gradients of acetonitrile and water. However, when acetonitrile is replaced by methanol, complete elution of PPGs with higher M_r is accomplished, but the oligomers merge into an unresolved signal envelope. This is attributed to a better solvation of the polyether backbone by methanol compared with acetonitrile due to hydrogen bonding between the ether oxygens and the hydroxy function of methanol and therefore, interactions with the hydrophobic stationary phase are efficiently counterbalanced. The obvious 'sticking effect' of high M_r PPG samples onto a C_{18} column can also be overcome by stationary phases with lower carbon content, such as, e.g., C_8 and C_4 matrices with acetonitrile as well as methanol as the organic modifier, both showing comparable efficiency. Alternatively, THF can be used as a 'solubility enhancer' in combination with acetonitrile for separation of either PPGs or PBGs on C_{18} stationary phases.

Conversion of the native PPG amines (Figure 1b) into their acetamide or 2,4-dinitrofluorobenzene (DNFB) derivatives (**Figure 11**) prior to chromatography, which is readily achieved with pyridine–acetic acid anhydride (1 : 1, v/v) or DNFB, respectively, is required to avoid interactions with residual silanols. Alternatively, the unmodified amines can also be separated with trifluoroacetic acid (TFA) as a mobile

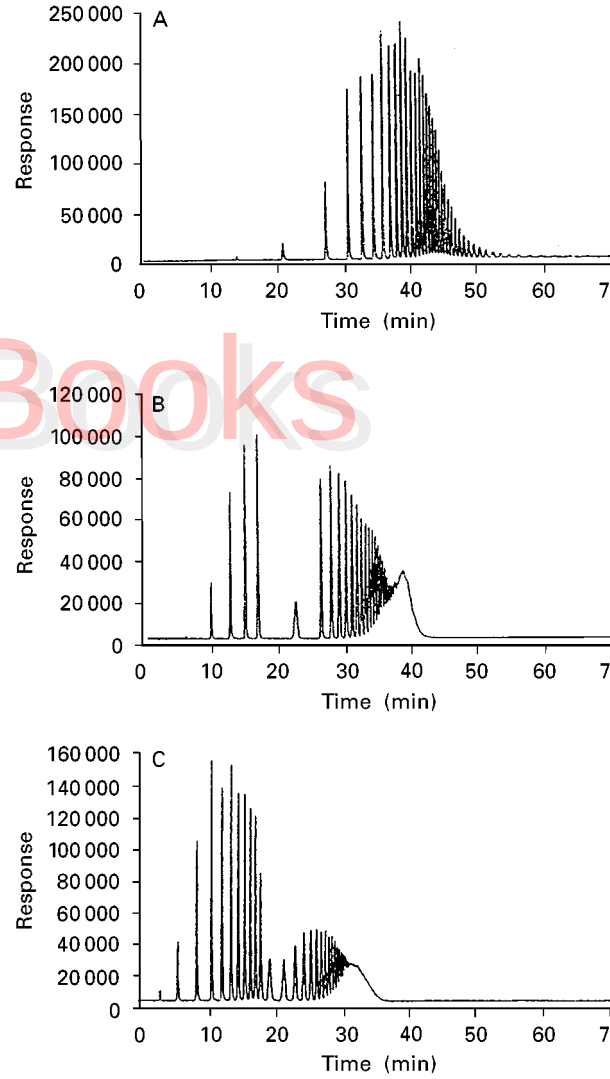

Figure 12 HPLC of PBG 1000 on a C_{18} column with (A) methanol, (B) ethanol and (C) 2-propanol as the organic modifier. (Reproduced from Rissler K, Fuchslueger U and Grether H-J (1993) *Journal of Chromatography* 654: 309–314, with permission from Elsevier Science.)

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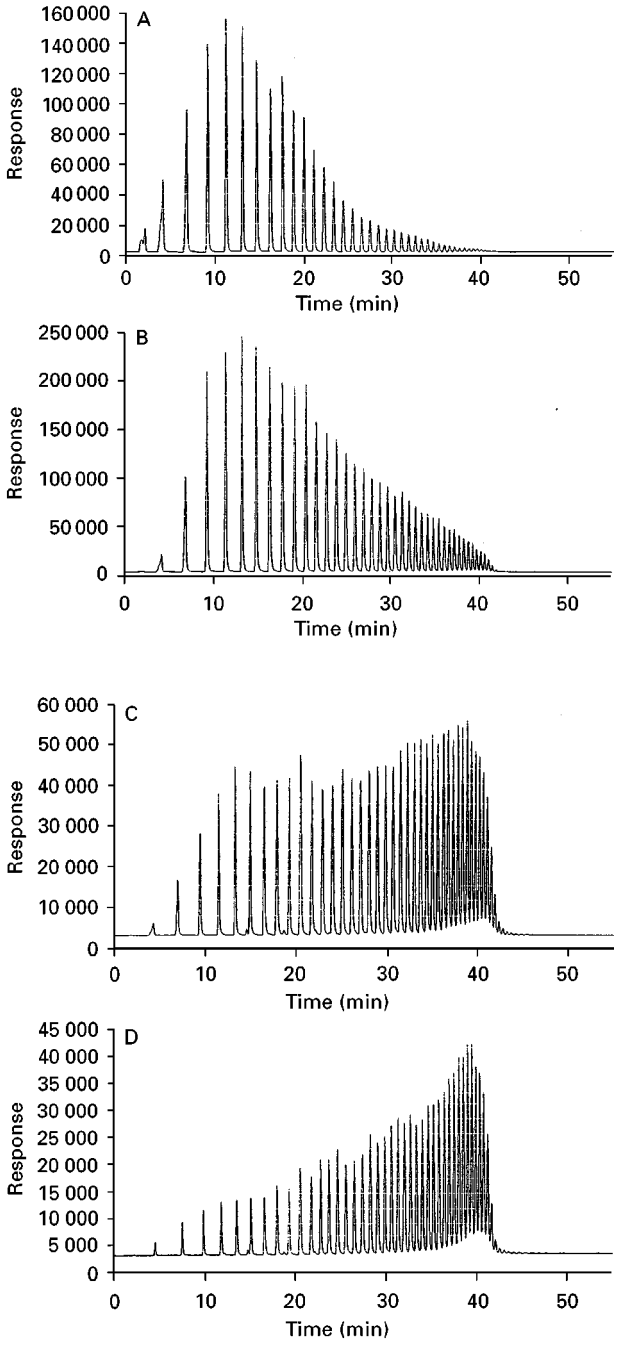

Figure 13 Chromatograms of (A) PBG 650, (B) PBG 1000, (C) PBG 2000 (D) PBG 3000 on a C_4 column and acetonitrile as organic modifier. (Reproduced from Rissler K and Fuchslueger U (1994) *Journal of Liquid Chromatography* 17: 2791–2808.)

phase additive for suppression of silanophilic interactions, but signal resolution is lower compared with the corresponding acetamides and DNFB derivatives.

PBGs exhibit still more pronounced solute–matrix interactions and so PBG 650 (average M_r 650) does not completely elute from a C_{18} matrix with a binary gradient of acetonitrile and water. In contrast, complete elution as well as excellent peak resolution is achieved on a C_{18} column with methanol as the organic modifier, whereas under these conditions, substantial amounts of PBG 1000 still remain on the stationary phase. A further change of organic modifier to ethanol or isopropanol affords complete elution of PPG 1000 (**Figure 12**), PBG 2000 and PBG 3000, but only the low-to-medium M_r oligomers are sufficiently separated, whereas the higher M_r sample constituents merge into a broad and unresolved signal. Presumably the same solvation effect as postulated for the PPGs also holds true.

Either excellent separation or complete elution is achieved for PBG 650, PBG 1000, PBG 2000 and PBG 3000 on a C_4 matrix with a binary gradient of acetonitrile and water and up to about 60 oligomers are observed (**Figure 13**). Although a column with markedly lower hydrophobicity compared with C_{18} and C_8 stationary phases is used, interactions of the substantially more hydrophobic PBG samples compared with PPGs are sufficient to give good resolution.

Underivatized PEGs, PPGs and PBGs are eluted in the range of increasing M_r, due to their continuously increasing interactive surface, whereas in the case of PEGs, the converse is true for the corresponding alkyl- and/or arylalkyl-substituted derivatives as a consequence of the dominant role of the lipophilic substituent on solute–stationary phase interactions.

Both signal monitoring by UV and ELSD can be applied and in particular ELSD is the method of choice for the native polyethers when sensitivity is not crucial, because it is at least one order of magnitude less sensitive than UV detection. Therefore, measurement of low amounts of polyethers requires derivatization with a chromophor or fluorophor. However, reaction with aromatic moieties has a dramatic influence on signal resolution of PEGs, which almost completely vanishes, whereas in contrast, PPG (**Figure 14**) and PBG (**Figure 15**) oligomers are still well resolved after conversion to their 3,5-dinitrobenzoyl derivatives. PPG amines having been reacted to the corresponding acetamides (DNFB derivatives) can be measured by either UV detection at 210 (355) nm or ELSD.

Unlike a UV detector, where concentration-dependent responses are measured and for this reason, calculation of response factors for the different oligomers is required, ELSD is a typical mass detection system like a refractive index detector. As a consequence, the signal intensities reflect the true mass distribution of oligomers. Moreover, ELSD offers an advantage over UV detection because it allows the use of solvents strongly absorbing in the usual UV range, such as, e.g., acetone and methylethylketone, and

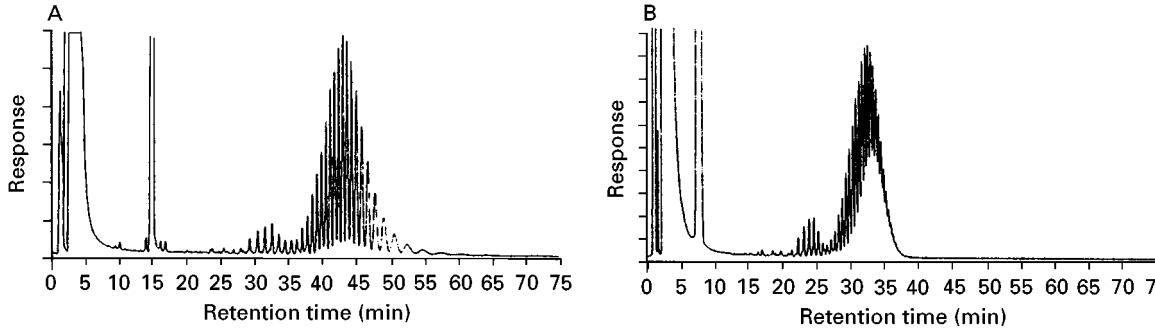

Figure 14 Chromatograms with PPG-1200 after derivatization with DNBCl and acetonitrile as organic solvent. (A) C_{18}; (B) C_8. (Reproduced from Rissler K, Künzi H-P and Grether H-J (1993) *Journal of Chromatography* 635: 89–101, with permission from Elsevier Science.)

furthermore, no baseline drift is observed with gradient elution.

Dual detection offers a further powerful tool in polymer separation and measurement of both UV and ELSD responses allow differentiation of, e.g. polyethoxylates containing either aromatic or aliphatic end groups.

During the past few years HPLC-mass spectrometry (LC-MS) has gained increased interest providing exact structural information and opening an additional dimension for polymer characterization. In contrast, matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI–TOF/MS) is still used off-line and is excellently suited for M_r and end group determination.

'Pseudo Reversed-Phase' Liquid Chromatography (pseudo RP-HPLC)

In contrast to their use in classical NP-HPLC with pure organic solvents of increasing polarity, bare silica gel stationary phases can also be operated with aqueous organic solvents of decreasing polarity, typically encountered in RP-HPLC. This new technique, termed 'pseudo reversed-phase' HPLC, is gaining more and more importance for polyether characterization.

Both native PEGs or their alkyl/arylalkyl derivatives (**Figure 16**) are efficiently separated according to the number of repeat units and in particular, much better oligomer resolution of the former compounds is achievable compared with RP-HPLC. Exceptions are RP-HPLC on an NH_2-bonded phase and ion exchange chromatography. However, despite this great advantage, retention rapidly increases with increasing M_r, but PEG 3000 is still separated into the maximum number of oligomers with an eluent system consisting of acetonitrile and water containing THF as the 'solubility modifier', as confirmed by MALDI–TOF/MS after conversion to its α,ω-bis(naphthylurethane) derivative (**Figure 17**). PEG samples with $M_r > 3000$ are strongly adsorbed onto the polar column matrix and can only be released from it with THF as the mobile phase modifier, but resolution vanishes completely.

Ion-exchange Chromatography (IEC)

Although lacking any ionizable groups, PEGs and their alkyl and/or arylalkyl derivatives can be separated by IEC, which is attributable to the unique properties of the 1,2-dioxoethylene structural units, being able to form five-membered cyclic complexes with alkali metal ions, such as K^+. Separation of PEG

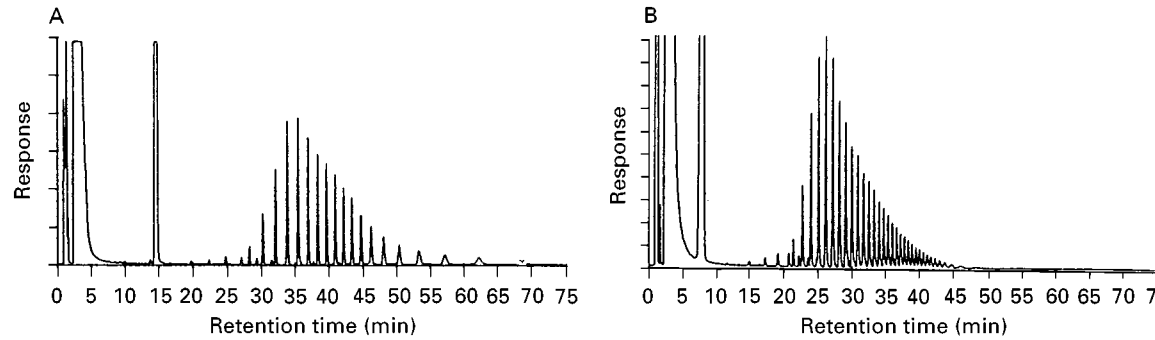

Figure 15 Chromatograms with PBG-1000 after derivatization with DNBCl and acetonitrile as organic solvent. (A) C_{18}; (B) C_8. (Reproduced from Rissler K, Künzi H-P and Grether H-J (1993) *Journal of Chromatography* 635: 89–101, with permission from Elsevier Science.)

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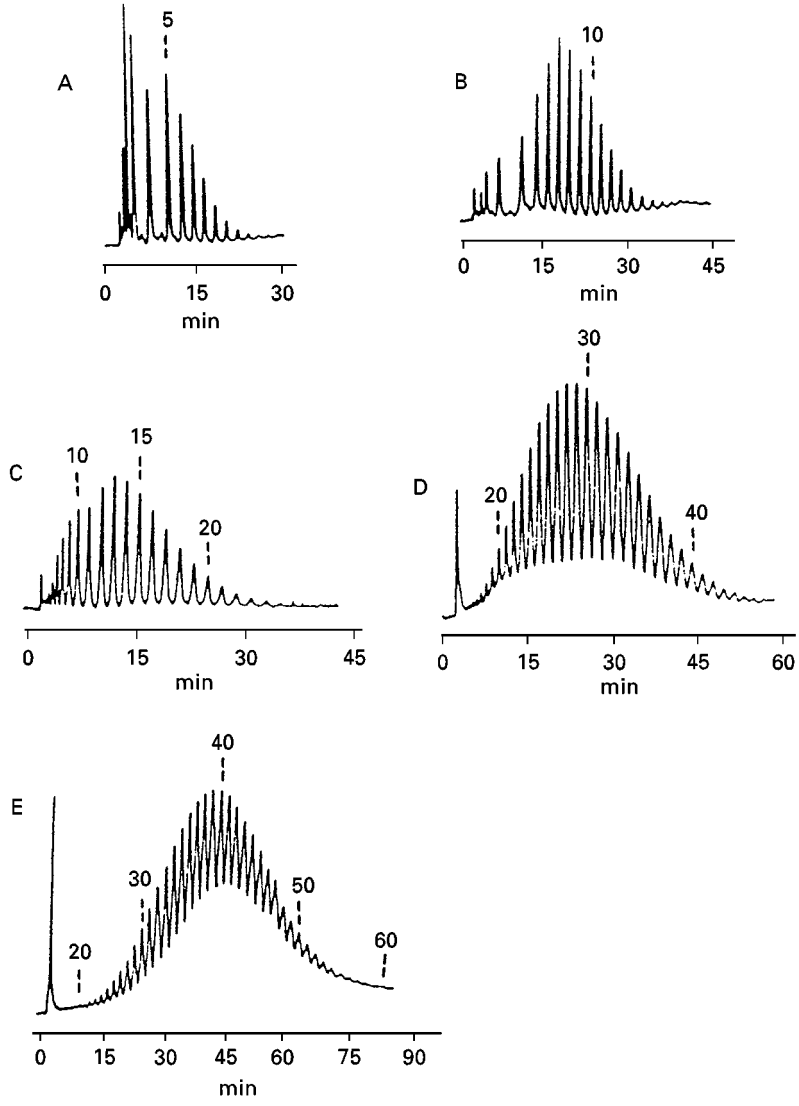

Figure 16 HPLC chromatograms of Synperonic NPs using gradient programme: (A) Synperonic NP6; (B) Synperonic NP10; (C) Synperonic NP15; (D) Synperonic NP30; (E) Synperonic NP40. Conditions: column, 5-μm Spherisorb NH_2 (250 × 4.6 mm I.D.). (Reproduced from Sun C, Baird M, Anderson HA and Brydon DL (1996) *Journal of Chromatography A* 731: 161–169, with permission from Elsevier Science.)

is achieved on either typical polymer-based cation exchangers (**Figure 18**) or on easily ionizable weakly basic stationary phases, such as NH_2. In the latter case, retention occurs by complexation of PEG at the ammonium sites of the stationary phase, being protonated by use of a slightly acidic mobile phase, and competitive displacement of the solute with increasing concentrations of K^+ ions in the eluent. Optimum results are obtained with potassium perchlorate in methanol and resolution is achieved according to both hydrophobic moiety and number of repeat units. As expected, retention increases with increasing number of ethoxymers, but this effect is so large that already PEGs of $M_r \cong 2000$ are almost irreversibly retained on the column.

Liquid Chromatography under Critical Conditions (LCCC)

LCCC is a typical method used in polymer analysis as the first step of 'two-dimensional' chromatography. It is applicable in either normal or reversed-phase LC modes and separates mixtures of polymers according to their different chemical composition. In contrast to HPLC of polymers, requiring gradient conditions for efficient separation of homologues, LCCC is an isocratic technique.

In general, LC of polymers is governed by (i) exclusion, (ii) solubility and (iii) adsorption. Depending on both temperature and composition of the solvents and nonsolvents used all three modes can occur. In

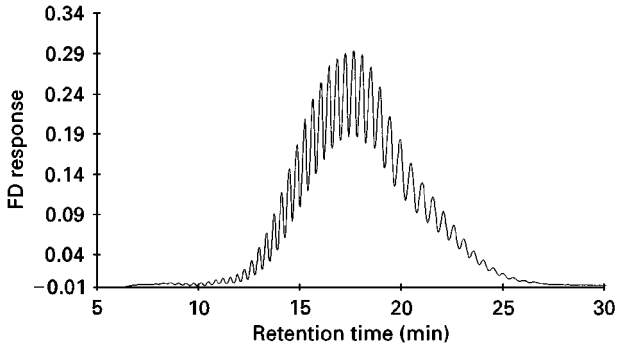

Figure 17 Chromatogram obtained from 100 ppm of the α,ω-bis(1-naphthylurethane) derivative of PEG 3000 dissolved in 10 μL THF on a Spherisorb Si 80 column (125 × 4.6 mm, 5 μm) with a ternary gradient of acetonitrile, water and THF (20% acetonitrile–80% water to 80% acetonitrile–20% THF in 40 min, followed by a drop to the starting conditions within 1 min and re-equilibration for 14 min (Reproduced from Rissler K, Wyttenbach N, Börnsen KO (2000) *Journal of Chromatography A*, in press, with permission from Elsevier Science.)

size exclusion a strong eluent is used, which ideally prevents enthalpic solute–stationary phase interactions affecting elution of sample constituents in the range of decreasing M_r. When the percentage of nonsolvent is raised, retention increases and changes more and more from pure size exclusion to precipitation or adsorption. A further increase of the percentage of nonsolvent affords pronounced solute–stationary phase interactions, resulting in separation of molecules proportional to their M_r. LCCC marks the transition point between size exclusion and adsorption. At this critical point of adsorption, complete compensation of the enthalpic as well as entropic terms of the solute's adsorption occurs. If polymers possessing different end groups differ in molar mass but not in chemical structure of repeat units are separated, a nonsolvent–solvent ratio can be found, at which oligomers merge into a common peak, regardless of their M_r. In this case, separation is only achieved with respect of end group functionality, whereas molecular weight heterogeneity is not revealed (**Figure 19**). In a subsequent step, all components having identical end groups can be separated according to M_r by SEC.

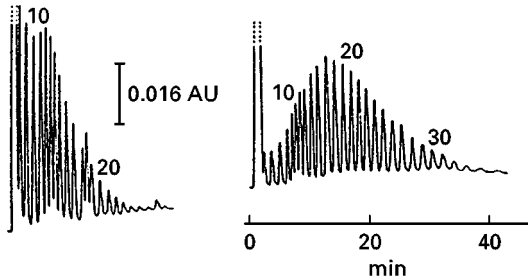

Figure 18 Typical separation in terms of POE chain distribution of POE(10)O (left) and POE(20)O (right). Mobile phase, methanol (0–3 min) to 7.5 mM KCl in methanol (30 min). Other conditions are given in the text. (Reproduced from Okada T (1992) *Journal of Chromatography* 609: 213–218, with permission from Elsevier Science.)

Future Developments

Owing to its excellent resolving power, capillary zone electrophoresis (CZE) may play an increasing role in polymer separation and recent applications seem to be promising. The chemistry of the inner surface of the capillaries ressembles that of open tubular HPLC and therefore, a wide variety of materials ranging

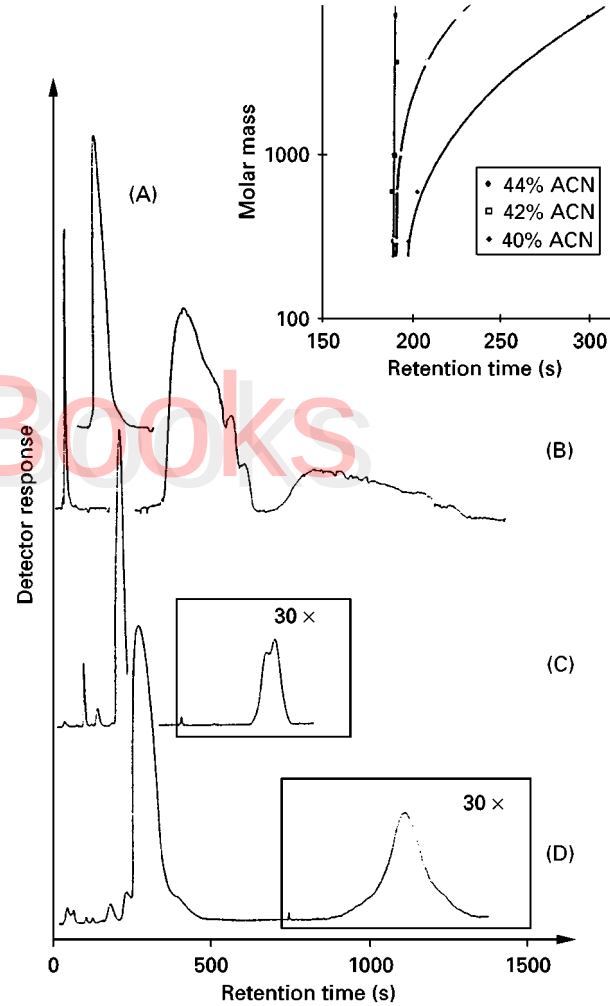

Figure 19 Critical diagram molar mass vs. retention time of polyethylene glycol (inset upper right corner) and chromatograms of functional PEOs at the critical point of adsorption of polyoxyethylene (PEO), stationary phase: Nucleosil RP-8, 60 × 4 mm I.D., solvent: acetonitrile–water 44 : 56% by volume, samples: C_{10}-PEO (A), C_{13},C_{15}-PEO (B), octylphenol-PEO (C), nonylphenol-PEO (D). ACN, acetonitrile. (Reproduced from Pasch H and Zammert I (1994) *Journal of Liquid Chromatography* 17: 3091–3108.)

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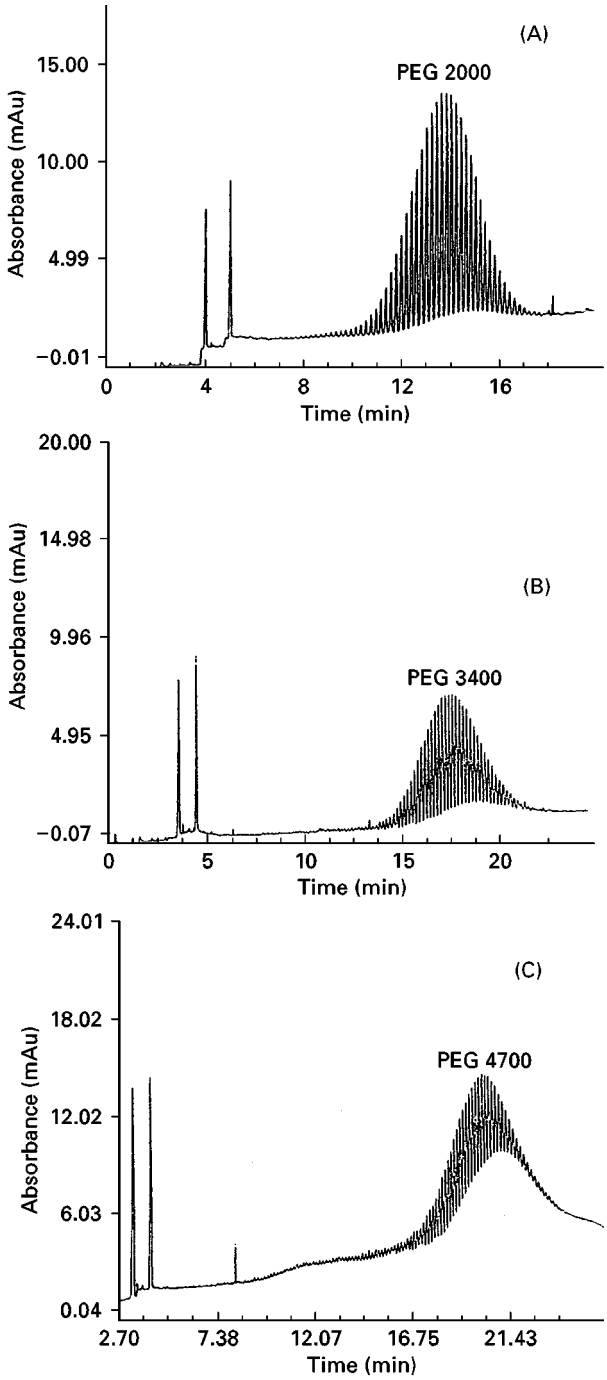

Figure 20 (A) Electropherogram of a PEG 2000 4% dextran solution. (B) Electropherogram of a PEG 3400 3% dextran solution. (C) Electropherogram of a PEG 4700 3% dextran solution. (Reproduced from Barry JP, Radtke DR, Carton WJ *et al.* (1998) *Journal of Chromatography A* 800: 13–19, with permission from Elsevier Science.)

from strongly polar (silica gel coated) to strongly hydrophobic (C_{18} coated) as well as gel-coated (e.g., PAGE) capillaries are available. Whereas CZE of polyethers as well as the NIS-based derivatives require derivatization with, e.g., phthalic anhydride or trimellitic acid anhydride yielding one and two free carboxy groups, respectively, per hydroxy function, the polyether amines are amenable to classical electrophoresis. Nevertheless, derivatization of neutral analytes with a charge-creating agent is not an ultimate prerequisite, because polymers can also be separated by micellar electrokinetic capillary chromatography (MEKC). Optimum separation efficiency of PEGs is achieved in the capillary gel electrophoretic mode after derivatization with trimellitic acid anhydride and the whole amount of oligomers can be separated up to M_r 5000, as confirmed by MALDI–TOF/MS investigations (**Figure 20**). As in HPLC, detection is performed by monitoring either UV response in the usual wavelength range down to about 200 nm or FD and depends on the chromophor introduced by derivatization.

At least at the moment, CZE affords separation of PEG oligomers up to $M_r \cong 5000$. However, signal resolution is not unlimited and markedly depends on the ratio $\Delta m/M$ (m = mass of repeat unit, M = total mass of polymer), i.e., the lower the ratio $\Delta m/M$ the lower the differences in the interactive surfaces between polymer $M(n)$ and $M(n+1)$. Fortunately, mass spectrometry, due to its unsurpassable resolution, still yields oligomer differentiation when separation into individual oligomers by chromatographic techniques fails. Nevertheless, prior chromatographic fractionation is essential to obtain clear and well-interpretable mass spectra. In this respect electrospray ionization (ESI) TOF/MS performed on-line and MALDI–TOF/MS carried out off-line with the isolated fractions are the methods of choice. Whereas polar polymers, such as, e.g., polyethers, polyacrylic acid and its esters are easily ionizable, addition of silver salts facilitates ionization of nonpolar analytes, such as, e.g., polystyrene, polybutadiene, bisphenol-A-diglycidylethers or phenol-novolaks.

In conclusion, it is expected that chromatography coupled to MS and therefore affording optimum structural information, will be dominant in future applications.

See also: **II/Chromatography: Liquid:** Enhanced Fluidity Liquid Chromatography; Mechanisms: Size Exclusion Chromatography. **III/Gradient Polymer Chromatography: Liquid Chromatography: Synthetic Polymers:** Liquid Chromatography.

Further Reading

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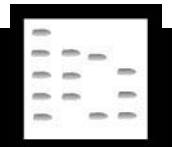
POLYMER ADDITIVES: SUPERCRITICAL FLUID CHROMATOGRAPHY



T. P. Hunt, ICI Technology, Middlesbrough, UK

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Introduction

Commercial polymers contain small quantities of low molecular weight additives which are evenly dispersed throughout the polymer matrix. They are typically present at concentrations in the order of 0.1–1.0% (w/w) but can be as high as 60% w/w in certain formulations. They make an important contribution to the properties and suitability of particular polymer grades.

The analysis of polymer additives is a two-stage process. The additives are first separated from the polymer by solvent extraction or reprecipitation. The extracted additives are then separated and quantified by a suitable chromatographic technique. This article is concerned with the application of supercritical fluid chromatography (SFC) to this second stage. However this also involves a discussion of coupled supercritical fluid chromatography–supercritical fluid extraction (SFE-SFC) in which both stages are combined into a single analysis.

Polymer Additives

The most common polymer additives are stabilizers, plasticizers, lubricants and flame retardants. Stabilizers are added to prolong the useful life of a polymer formulation by protecting it from thermal and light-assisted oxidation. This process is caused by the formation in the polymer chain of free radical sites which can react with oxygen to form unstable peroxy radicals and ultimately cause polymer chain scission. Stabilizers are divided into four main classes: UV absorbers, primary antioxidants, secondary antioxidants and quenchers.

UV absorbers such as benzophenones and triazoles screen the polymer from harmful photons by absorbing them and then dissipating the excitation energy as heat so there is no radical formation. Primary antioxidants are typically hindered phenols. They react with free radicals to prevent further propagation. Secondary antioxidants destroy the hydroperoxide sites on the polymer chain which could otherwise be converted to peroxy radicals. They tend to be sulfur- or phosphorus-containing compounds. Quenchers are usually organonickel compounds and their function is to take over the energy absorbed by the chromophores in the polymer and dissipate it as heat.

Lubricants are added to make the polymer easier to process by controlling the melt rheology during thermoplastic moulding. They optimize the properties of the finished article to create smooth and unblemished surfaces and minimize stress fractures. External lubricants are compounds that are added to a polymer blend to control the degree of adhesion and friction between the polymer melt and hot processing equipment. Internal lubricants are added to polymer blends to reduce the melt viscosity to facilitate lower processing temperatures and to improve heat dissipation. Many lubricants posses a combination of internal and external characteristics. Lubricants are typically fatty alcohols, acids and esters and hydrocarbon waxes.

Plasticizers are high-boiling, organic chemicals which are often present at high concentrations, solvating the polymer chains to form stable gels. As a result,

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